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Homolaphlyctis polyrhiza gen. et sp. nov., a species in the *Rhizophydiales* (*Chytridiomycetes*) with multiple rhizoidal axes

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Abstract — An undescribed cellulosic chytrid with multiple rhizoidal axes, JEL142, has grouped in molecular hypotheses with *Batrachochytrium dendrobatidis*, the chytrid pathogen of amphibians, and thus is of interest for genetic and physiological comparisons. To describe this member of the *Rhizophydiales*, we examined its zoospore ultrastructure and developmental morphology. Based on a reanalysis of rDNA data plus ultrastructural and morphological characters, we name this fungus *Homolaphlyctis polyrhiza* gen. et sp. nov.

Key words — *Chytridiomycota*, phylogeny, *Rhizophlyctis*, TEM

Introduction

A chytrid referred to by its isolate number, JEL142, has been in phylogenies since the first molecular hypotheses of the *Chytridiomycota* (James et al. 2000, 2006; Letcher et al. 2008b) but has not been identified to species or received a formal name. This isolate is of interest because it groups as sister to *Batrachochytrium dendrobatidis* Longcore et al. (Longcore et al. 1999), the pathogen of amphibians, and because it is one of the few members of the *Rhizophydiales* Letcher (Letcher et al. 2006) that have thalli with multiple rhizoidal axes. Although the phylogenetic distance based on branch lengths in phylograms is large and support values for the relationship have been low (James et al. 2006; Letcher et al. 2008b), JEL142 seems to be the closest relative to *B. dendrobatidis* now in culture. In an effort to identify genes that enable

B. dendrobatidis to be pathogenic, the genome of JEL142 has been sequenced for comparison (Joneson et al. 2011). In this paper, we reanalyze the phylogenetic placement of JEL142 and provide a description of the ultrastructure of its zoospore and its developmental morphology. These analyses place isolate JEL142 in the *Rhizophydiales,* but outside of described families or genera. Therefore we describe a new genus and species to accommodate isolate JEL142.

Materials & methods

We first observed this chytrid on onionskin bait (outer, dry bulb scales of white onions) placed in a gross culture containing the aquatic plant *Eriocaulon aquaticum*, associated debris and lake water in a deep Petri dish. The collection site was Mud Pond, a small (1.7 ha), acidic (pH 4.6), oligotrophic and fishless lake in Hancock County, Maine (Davis et al. 1994, Rhodes & Davis 1995). With standard methods (Barr 1986) we isolated JEL142 into pure culture on modified PmTG nutrient agar (Longcore 1992). Since initial isolation in 1994 this chytrid has grown on nutrient agar slants with transfers at three-month intervals and storage at ~4 °C after growth. We collected zoospores from growth on 20 mPmTG plates and fixed them for TEM with s-collidine buffered glutaraldehyde followed by osmium tetroxide. Zoospores were rinsed, added to agar and en bloc stained with uranyl acetate before embedment in epon araldite plastic (Barr 1981, Longcore 1992). Sections were examined at 60 kV. We photographed developmental morphology of thalli grown on mPmTG nutrient agar with a Spot RT digital camera. Phylogenetic analysis of *Rhizophydiales* was conducted using the initial alignments of 18S, 28S, and 5.8S rRNA subunits produced by James et al. (2006). Additional sequences were added from Letcher et al. (2004, 2008b), and the alignment manually adjusted in MacClade 4.08 (Maddison & Maddison 1992). The phylogeny was estimated using maximum likelihood in RAxML-7.0.4 (Stamatakis 2006) with 100 heuristic searches beginning from random trees. Support for nodes was established by bootstrapping in RAxML (1000 pseudo-replicates) and Bayesian posterior probabilities in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001).

Results

The phylogenetic analysis of *Rhizophydiales* based on combined 18S, 28S, and 5.8S subunits provides strong support, 77 bootstrap percentage and 0.99 posterior probability, for a sister relationship between *H. polyrhiza* and *B. dendrobatidis* (Fig. 1). These two species group with a strain identified as *Entophlyctis helioformis* (JEL326), and this clade is sister to the other members of the *Rhizophydiales*. As seen in previous studies, the phylogenetic distance between *H. polyrhiza* and *B. dendrobatidis* is rather large as suggested by branch lengths, and the percentage difference between rRNA sequences of the two species is ~10%.

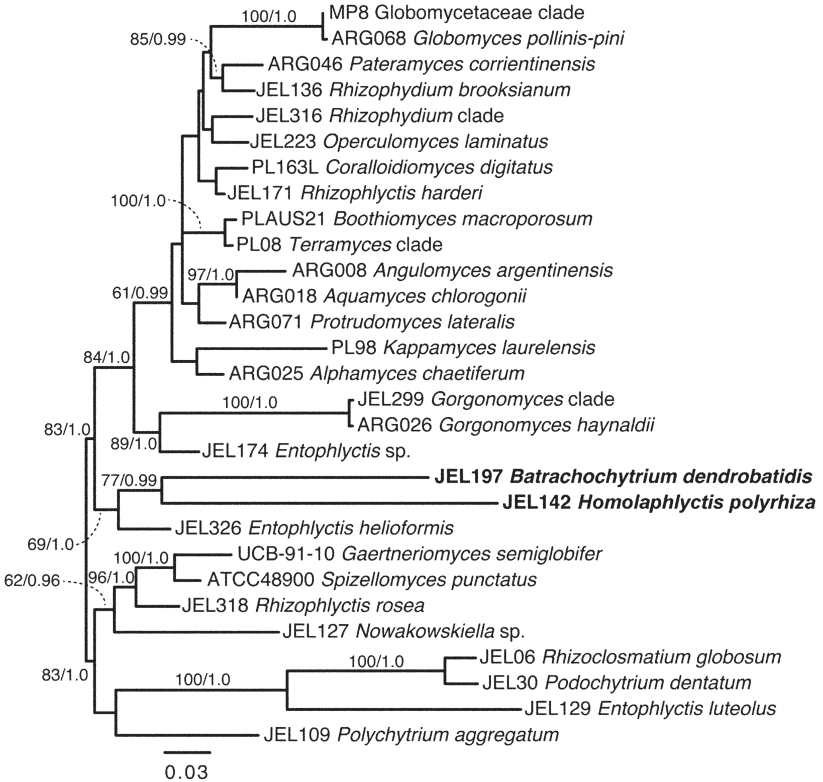

Figure 1. Phylogeny of *Rhizophydiales* showing sister relationship between *Homolaphlyctis polyrhiza* and *Batrachochytrium dendrobatidis*. The phylogeny was based on a combined analysis of 18S, 28S and 5.8S ribosomal RNA subunits and was estimated using maximum likelihood under a GTR+I+Γ model of evolution with RAxML-7.0.4 (Stamatakis 2006). Values at nodes indicate support as estimated by maximum likelihood from 1,000 bootstrap pseudo-replicates followed by Bayesian posterior probabilities. Only nodes receiving >60% bootstrap support are labeled. Further strain details and GenBank accession numbers can be found in James et al. (2006) and Letcher et al. (2004, 2008b).

Taxonomy

Homolaphlyctis Longcore, Letcher & T.Y. James, **gen. nov.**

MycoBank MB561579

Zoosporarum ultrastructura Rhizophydialium characteristica, constans ex microtubulis separatis duo vel tres e kinetosomate ad rumposoma in globulo solitario extensorum; microcorpus in latere interiore globuli lipoidei. Calcar kinetosomale et zona vesiculata

circum kinetosoma absunt. Mitochondrion lobatum. Vesiculae corde denso per cytoplasma dispersae.

Type species: *Homolaphlyctis. polyrhiza* Longcore, Letcher & T.Y. James

Etymology: Named for R.L. Homola in whose laboratory this work began, and A.D. Homola, who helped collect the sample.

Zoospore ultrastructure typical for members of the *Rhizophydiales*, consisting of stack of 2–3 separate microtubules leading from the kinetosome to a rumposome on a single lipid globule; microbody on inner side of lipid. Lacking kinetosome-associated spur and vesiculated area around kinetosome. Mitochondrion lobed. Dense-cored vesicles throughout cytoplasm.

Homolaphlyctis polyrhiza Longcore, Letcher & T.Y. James, **sp. nov.** Figs. 2, 3

MycoBank MB561580

Thallus axibus rhizoidalibus tres vel pluris extense dispersis. Papillae evacuationis tubulosae numerosae, breves usque ad longae.

Type: Fig. 2a–h **(holotype)** photographed from pure culture JEL142 isolated from cellulosic substrate placed with a collection of *Eriocaulon aquaticum* collected from Mud Pond, (44°38′00.26″N 68°05′15.81″W) Hancock County, Maine on 15 April 1994. [GenBank DNA sequences: AH009051 (SSU rDNA); EF634247 (LSU rDNA); EF634249 (ITS1-5.8S-ITS2).]

Etymology: The epithet refers to the multiple rhizoidal axes.

Thallus with three or more widely distributed rhizoidal axes. Multiple, short to long tubular discharge papillae.

Expanded Description — The generation time for *H. polyrhiza* on mPmTG nutrient agar is 2–3 days at 23 °C. In motion, zoospores are spherical (Fig. 2h, arrowhead) and contain a single lipid globule near the base of the flagellum (Fig. 2a). They encyst and within 24 h form a thallus with multiple rhizoidal axes (Fig. 2b). At maturity rhizoids can extend to more than twice the diameter of the zoosporangium (Fig. 2c); distally rhizoids are fine (<0.5 μm) and isodiametric, but near the thallus they are slightly swollen (Fig. 2d). Zoosporangia form discharge papillae (Figs. 2e, f) within 52 hr, with the number of papillae increasing with size of the zoosporangia. Papillae may be barely raised (Fig. 2f), short tubes (Fig. 2e) or, in crowded conditions, long tubes (Fig. 2g). Zoospores are elongate when discharged (arrows, Fig. 2h) but soon become spherical (dia 3.5–4.5 μm with flagellum up to 28 μm).

Ultrastructural characters — The ultrastructure (Figs 3a–e) of the *H. polyrhiza* zoospore is nearly identical to Barr's illustration of a typical *Rhizophydium* zoospore (Barr & Hadland-Hartmann 1978). A membrane-bound aggregation of ribosomes is in the center of the zoospore, outside of which are the nucleus, a single lobed mitochondrion, and a single lipid globule (Figs. 3a, b). A lobe of a mitochondrion is often anterior to the kinetosome

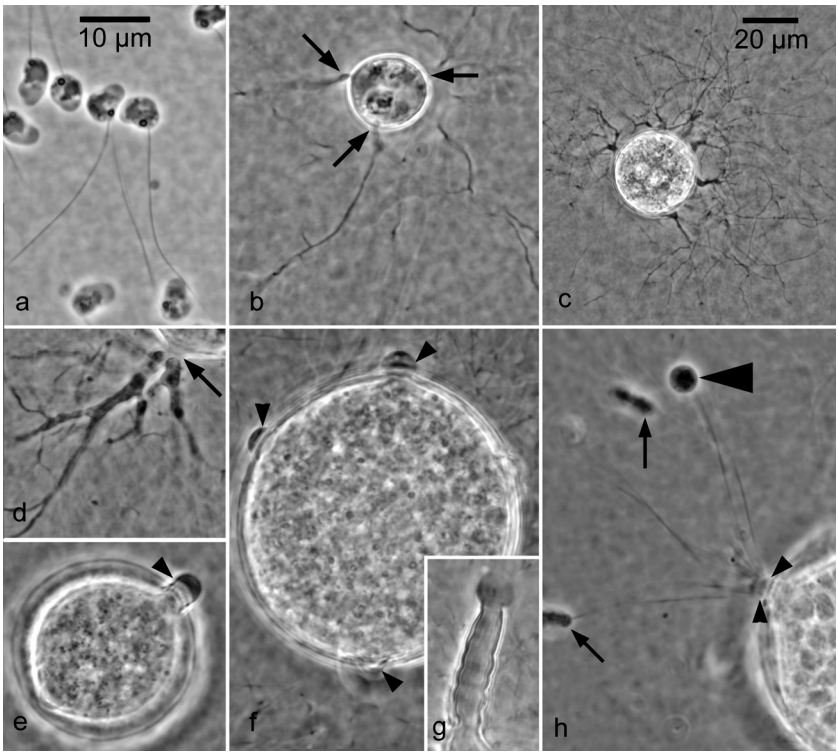

Figure 2. Developmental morphology of *Homolaphlyctis polyrhiza* in pure culture on mPmTG nutrient agar. a. Zoospores with single lipid globule, aggregated ribosomes and single posteriorly directed flagellum. b. Young thallus with multiple rhizoidal axes (arrows). c. Nearly mature thallus showing the extent of rhizoids. d. Rhizoidal bases (arrow) are enlarged. e. Papilla (arrowhead) on nearly mature zoosporangium. f. Short, evenly spaced discharge papillae (arrowheads). g. Long discharge papilla, nearly ready to release zoospores. Note breakdown of apical, hyaline papillar material. h. Zoospores (arrows) elongate during discharge through papillar opening (arrowheads) but become spherical shortly after release (large arrowhead). Scale bar in a, for all figures except c.

(Fig. 3a). The microbody is appressed to the side of the lipid globule toward the center of the zoospore (Fig. 3b), and a rumposome (= fenestrated cisterna of Letcher et al. 2004) is appressed to the lipid near the surface of the cell (Fig. 3b). The non-flagellated centriole is parallel to the kinetosome and joined to it by a bridge of partly overlapping fibers centrally located between the two structures (Figs. 3c, d; arrow); the fibrils form a wide (>0.075 µm) zone of convergence. The non-flagellated centriole is shorter than its width (Figs. 3c, e). A microtubular root, composed of separate, parallel microtubules, extends from the side of the kinetosome to the rumposome (Fig. 3e). A terminal plate is in the lumen of

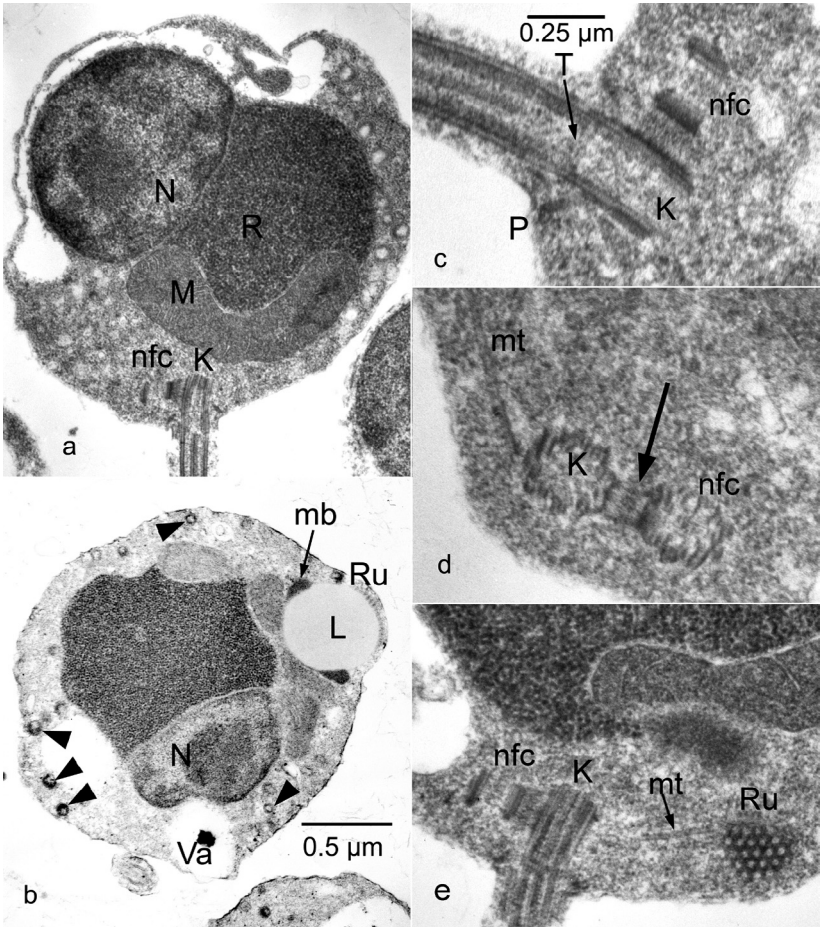

Figure 3. Ultrastructural features of *Homolaphlyctis polyrhiza* zoospores. a. Longitudinal section of zoospore with nucleus (N); aggregated ribosomes (R); lobed mitochondrion (M); kinetosome (K); non-flagellated centriole (nfc). b. Transverse section with lipid globule (L); microbody (mb); rumposome (Ru); vacuole (Va); and vesicles (arrowheads). c. Longitudinal section of kinetosomal region with kinetosomal props (P) and terminal plate (T). d. Cross section of kinetosome and non-flagellated centriole with fibrillar connection and wide zone of convergence (arrow) between the two structures. Microtubule root (mt) extends from the kinetosome. e. Longitudinal section through the kinetosome and non-flagellated centriole; microtubules extend from the kinetosome to the rumposome, shown in face view. Scale bar in b also for a; scale bar in c also for d–e.

the axoneme (Fig. 3c). Typical for members of the *Rhizophydiales* (Letcher et al. 2006), a flagellar plug is absent from the transition region of the flagellum. In the peripheral cytoplasm outside the central core are numerous, large

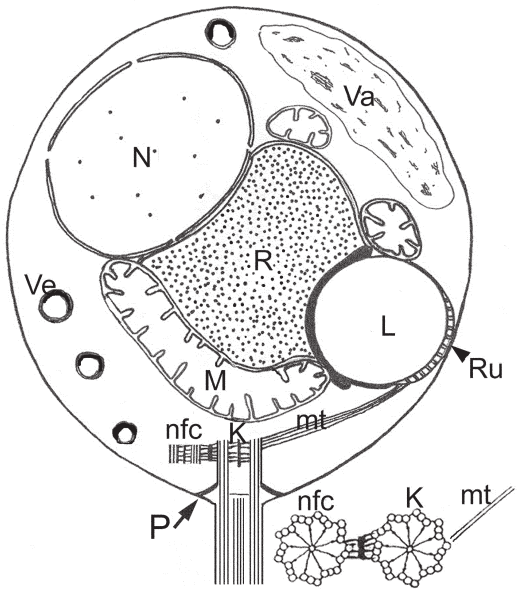

Figure 4. Summary diagram of zoospore ultrastructure of *Homolaphlyctis polyrhiza*. Kinetosome (K); lipid globule (L); mitochondrion (M); microbody (mb); microtubule root (mt); nucleus (N); nonflagellated centriole (nfc); prop (P); vacuole (Va); vesicle (Ve).

(~200 nm diameter) cored vesicles (Fig. 3b; arrowheads); a large vacuole is often present. Ultrastructural features are summarized in Fig. 4.

Comments — We found this saprobic organism only once, from an oligotrophic, acidic lake. Based on descriptions of genera of the *Chytridiales* sensu Sparrow (1960), it would have been placed in the genus *Rhizophlyctis* A. Fisch. because it develops endogenously and has multiple rhizoidal axes. Molecular and TEM evidence have altered our concept of the importance of morphological characters (e.g., James et al. 2000, 2006; Mozley-Standridge et al. 2009) and chytrids with endogenous development and multiple rhizoidal axes occur not only in the *Rhizophlyctidales* (Letcher et al. 2008a) but also other orders of the *Chytridiomycetes*, now including the *Rhizophydiales*.

Acknowledgments

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